

ImmunoPure™ HD



Normal Human CD8+ Cytotoxic T Cells

A division of Gene Therapy Systems, Inc.

Catalog #	Description/Content	Amount
PBMC004A	Normal Human CD8+ Cytotoxic T Cells (negatively selected), Cryopreserved	10.0 x 10 ⁶ cells

Shipping and Storage:	Cells are shipped on dry ice. <u>For maximum viability, it is best to use cells as soon as possible.</u> For short term (3 weeks or less) store cells at -80°C; for longer term, store cells in liquid nitrogen (-170°C). NOTE: viability cannot be guaranteed following -170°C storage or beyond one week at -80°C.
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Related Products	Catalog Numbers
Normal Human Peripheral Blood Mononuclear Cells	PBMC000 (5.0x10 ⁶); PBMC001 (25.0x10 ⁶); PBMC002 (75.0x10 ⁶).
Normal Human CD4+ Helper T Cells	PBMC003A (10.0x10 ⁶).
Normal Human CD14+ Monocytes	PBMC005A (10.0x10 ⁶).
Normal Human CD19+ B Cells	PBMC006A (10.0x10 ⁶).
Normal Human CD56+ Natural Killer Cells	PBMC007A (10.0x10 ⁶).
Normal Human Dendritic Cells	PBMC008A (0.5x10 ⁶).

INTRODUCTION

Normal Human CD8+ Cytotoxic T Cells are a sub-group of T lymphocytes capable of inducing the death of infected -with viruses or other pathogens- somatic or tumor cells. Most CD8+ Cytotoxic T cells express T-cell receptors that can recognize a specific antigenic peptide bound to Class I MHC molecules (present on all nucleated cells) and a glycoprotein called CD8. The MHC molecule keeps the Cytotoxic T cell and the target cell bound closely together during antigen-specific activation. CD8+ T cells are recognized as Cytotoxic T cells once they become activated and are generally classified as having a pre-defined cytotoxic role within the immune system. The ImmunoPure HD CD8+ Cytotoxic T Cells are isolated from fresh human adult peripheral blood of healthy donors at IRB-approved and FDA-licensed blood banks, using apheresis and immunomagnetic separation techniques.

MATERIALS AND METHODS

A. General Medium Requirement

RPMI 1640 medium + 10% (v/v) FBS, 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, 50 U/ml penicillin, 50 mg/ml streptomycin, and 50 mg/ml kanamycin.

B. Thawing and Culturing Cells

1. Prepare a 37°C water bath to temperature.
2. Keep all samples frozen until the bath is ready.
3. Place the vials into the water bath, being careful not to submerge below the junction between the lid and the vial.
4. Place vial of cells in 37°C water bath and agitate until thawed. It is important to thaw the cells quickly; do **NOT** allow thawed cells to remain in freezing media any longer than necessary.
5. When only a small amount of ice remains, remove the vials and dry with a lab tissue. Clean the top of the vial with a lab tissue wetted with 70% alcohol; **avoid wiping away the labeling.**
6. Within about 30 sec., slowly add one milliliter of medium (containing serum) to the thawed cells.

7. Slowly add thawed cells to 8 ml of medium containing serum. Invert tube 2 or 3 times to mix or mix gently by pipetting up and down several times.
8. Centrifuge for 5 min at 400 x g.
9. Aspirate or decant the supernatant and gently resuspend the cell pellet in 10 ml of medium.
10. Remove an aliquot for cell count and proceed with experimental manipulations.
11. Culture the cells in RPMI 1640 medium supplemented with 10% (v/v) FBS, 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, 50 U/ml penicillin, 50 mg/ml streptomycin, and 50 mg/ml kanamycin at a density of 1-2 million cells/ml.

NOTE: The cell suspension may form clumps after standing at room temperature. This can be avoided by preparing and using the cells promptly or by adding DNase to the suspension at a final concentration of 10 units per ml.

Normal Human CD8+ Cytotoxic T Cells, Manual

C. References

1. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. Ritu Sarin, Xingxin Wu, and Clara Abraham. *PNAS*, June 7, 2011, Vol. 108, No. 23, 9560–9565.

The purchase price paid for the ImmunoPure™ HD cells and reagents grants end users a non-transferable, non-exclusive license to use the kit and/or its components for **internal *in vitro* research use only** as described in this manual; in particular, “research use only” excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis. Separate licenses are available for non-research use or applications. **The PrimaPure™ HD cells and reagents are not to be used in human diagnostic or therapeutic applications, including primary or secondary use to produce or derive, directly or indirectly, any components used as drugs for human or animal use.** Although routinely tested for HIV-1, HBV, HCV, Syphilis, and other infectious diseases, these cells must be handled as potentially infectious. There is no test that can completely guarantee the absence of infectious agents; care and attention should be exercised in handling the ImmunoPure cells by following standardized research lab practices, wearing protective lab clothing, and using appropriate equipment.

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